Intradermal injection of umbilical cord mesenchymal stem cells was more effective than platelet rich plasma in increasing amount of collagen and fibroblasts in Wistar rats (*Rattus norvegicus*) back skin exposed to ultraviolet B rays

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Abstract

Background: Ultraviolet B exposure is one of the causes of extrinsic skin aging. Ultraviolet exposure may lead to skin collagen damage. This study about mesenchymal stem cells from umbilical cord (UCMSC) compared with platelet rich plasma (PRP) with the aim was to prove that the intradermal injection of UCMSC was more effective than PRP in increasing amount of collagen and fibroblasts in Wistar rats' back skin exposed to Ultraviolet B rays.

Methods: This study used post-test only control group design. Subjects were 36 Wistar rats aged 2.5 months. A control group consisting of 18 Wistar rats treated with intradermal injection of PRP and the treatment group with UCMSC. Both groups were exposed to UVB with a total radiation dose of 840 mJ/cm². The parameter of collagen was determined by picro sirius red staining while fibroblasts by Haematoxylin-Eosin staining.

Result: The mean amount of collagen in the control group was lower (57,82±6.52%) than the treatment group (65.69±4.51%) with p<0.001. The result showed there were differences in the number of collagen in both groups (p<0.05). The mean number of fibroblasts in the control group was lower (22.63±5.98 cell/field of view) than the treatment group (28.20±8.90 cell/field of view) with p=0.034. The result showed there were differences in mean of fibroblasts in both groups (p<0.05).

Conclusion: It can be concluded that the intradermal injection of UCMSC was more effective in increasing the amount of collagen and fibroblasts than intradermal injection of PRP in Wistar rats' back skin exposed to UVB rays.

Keywords: *Umbilical cord mesenchymal stem cell (UCMSC), Platelet rich plasma (PRP), UVB rays, Collagen, Fibroblasts*

Introduction

The aging process causes a gradual decrease in the ability to maintain homeostasis and regenerate all the tissues and organs of the body. Aging is influenced by intrinsic factors that

come from within the body, including race, genetics, hormonal processes, glycosylation, methylation, apoptosis, and decreased immune system. Extrinsic factors come from ultraviolet, environmental pollution, cigarette smoke pollution, unhealthy lifestyles, unhealthy diets, and stress.¹

One of the extrinsic factors that play a role in photoaging is exposure to ultraviolet rays, especially ultraviolet B rays. Repeated UV exposure will cause a formation of reactive oxygen species (ROS), which activates the cell surface receptors of epidermal growth factor (EGF), IL-I, insulin, keratinocyte growth factor, and tumor necrosis factor- α (TNF- α). These receptors stimulate the intercellular signaling pathways of mitogen-activated protein (MAP) kinase P38 and c-Jun amino-terminal kinase (JNK). The kinase activation further induces AP-1 nuclear transcription that increases MMP-1. Decreased levels of procollagen I due to increased expression of MMP-1 occur more in aging due to the UV exposure.²

Skin damage in photoaging can occur in the components of the epidermis, dermis, and skin appendages. One of the microscopic changes that occur in the dermis layer of the skin that is subjected to photoaging can be in the form of a significantly reduced number of collagen fibers. The damage will cause clinical signs such as; fine wrinkles, facial wrinkles, clearer lines of expression and even sagging of the skin.³

Umbilical cord mesenchymal stem cell (UCMSC) contains several growth factors such as TGF- β , vascular endothelial growth factor (VEGF), fibroblasts growth factor (FGF), hepatocyte growth factor (HGF), and as an antioxidant superoxide dismutase (SOD). In some studies, UCMSC is thought to be used as anti-aging because of several secretory factors in it. Also, it has a paracrine effect on human dermal fibroblasts (HDFs), as evidenced by the acceleration of wound healing by stem cells.⁴

Platelet-Rich Plasma (PRP) is a high concentration of platelet preparations obtained by centrifugation of peripheral blood. PRP can release growth factors that have a regulatory effect on biological processes at the cellular level, such as cell migration, cell proliferation, and cell differentiation. Platelets will release growth factors along with other cytokines from the granules when activated. It is widely known that PRP contains seven types of growth factors, namely: Platelet-Derived Growth Factor (PDGF), TGF- β 1, TGF- β 2, VEGF, and EGF.⁵ The purpose of this study was to prove that the intradermal injection of UCMSC was more effective than PRP in increasing amount of collagen and fibroblasts in Wistar rats' back skin exposed to Ultraviolet B rays to provide the effectiveness in preventing photoaging.

Methods

This study used a post-test only control group design.⁶ This study Involved 36 Wistar rats (*Rattus norvegicus*) aged 2.5 months and weighed 190-200 grams as the subjects. The Subjects were divided into two groups: a control group and the treatment group, both groups consisted of 18 Wistar rats (*Rattus norvegicus*). The control group treated with intradermal injection of PRP with wide 1x1 cm, needle 32G length 1mm with distance 3mm/0.1cc per injection in the back Wistar rats. The PRP derived from human blood were taken at PMI Jakarta, whereas for the treatment group treated with intradermal injection of UCMSC with wide 1x1 cm, needle 32G length 1mm with distance 3mm/0.1cc per injection with dose 0.8×10^6 cell in 1 cc per Wistar rats from Stem Cell & Cancer Institute, Kalbe Farma Tbk.

The intradermal injection was applied on the 8th day of the examination. Both groups were exposed to UVB with the total radiation dose was 840 mJ/cm² for four weeks (3 times per week). This study was held in Unit Laboratorium Biomedic Terpadu Medical Faculty, Udayana University and for the examination of hispatologists at Sentra Diagnostic Pathology, Bali. The observed parameter is the amount of collagen with Picro Sirius Red staining and the number of fibroblasts with Haematoxylin-Eosin staining.

Results

This study examined both PRP and UCMSC in increasing the amount of collagen and fibroblasts in Wistar rats. By using an Independent T-test, the result showed that the mean collagen in the control group was ($57.82 \pm 6.52\%$) and the collagen mean in the treatment group was ($65.69 \pm 4.51\%$). The mean fibroblasts in the control group was (22.63 ± 5.98 cell/field of view) and the mean fibroblasts in the treatment group was (28.20 ± 8.90 cell/field of view).

The results of both groups showed that the control group was lower than the treatment group in increasing the amount of collagen and the number of fibroblast in Wistar rats. The results of collagen in a control group was significantly lower ($57.82 \pm 6.52\%$) than the treatment group ($65.69 \pm 4.51\%$) with $p < 0.001$. The result showed there were differences in the number of collagen in both of groups ($p < 0.05$). Moreover, the mean number of fibroblasts in the control group was also significantly lower (22.63 ± 5.98 cell/field of view) than the treatment group (28.20 ± 8.90 cell/field of view) with $p = 0.034$. The result showed there were differences in mean of fibroblasts in both of groups ($p < 0.05$).

Figure 1. Collagen control group histology examination (Picro 100x). Description K9: control group (Picro 100x), white color: dermal tissue other than collagen tissue (49%), black color: collagen tissue (51%)

Figure 2. Collagen control group histology examination (Picro 100x). Description K9: control group (Picro 100x), white color: dermal tissue other than collagen tissue (49%), black color: collagen tissue (51%)

Figure 3. Inter-group histological examination of fibroblasts. Description K1.3: control group (H&E 400x), P1.3: treatment group (H&E 400x), the number of fibroblasts cell in the treatment group (P1.3) more than the control group (K1.3)

Figure 4. Comparison of control collagen (PRP) between treatment collagen (UCMSC)

Figure 5. Comparison of control fibroblasts (PRP) between treatment fibroblasts (UCMSC)

Discussion

The role of PRP and UCMSC in increasing amount of collagen and fibroblasts

In this study, exposure to UVB rays with a total dose of 840 mJ/cm² for 4 weeks was sufficient to cause damage to collagen and fibroblasts in the skin of the dermis of Wistar rats. Intradermal administration of UCMSC has provided a protective effect against damaged collagen tissue and fibroblasts of Wistar rats exposed to UVB light. That was evidenced by a significant increase in the amount of collagen and fibroblasts compared to the control group who has given an intradermal injection of PRP.

By administering an intradermal injection of both PRP and UCMSC, it will increase the expression of genes that play a role in repair processes, such as the TGF- β and platelet-derived growth factor (PDGF) that stimulate the fibroblasts to multiply, migrate and prevent damage to the extracellular matrix and inhibit emphasis on the expression of type I and type III procollagen genes. TGF- β inhibits the proliferation of macrophages and lymphocytes to inhibit the proinflammatory effect, suppresses the increase in nuclear factor-kappa beta (NF- κ B) therefore preventing the induction of IL-1, IL-6, VEGF, and TNF- α .⁷⁻⁸

TGF- β also inhibits AP-1 and MMP-1 by increasing the activity of proteinase inhibitors, namely tissue metalloproteinase inhibitors (TIMPs), therefore can prevent and reduce damage to collagen tissue, thus the collagen synthesis can increase. Fibroblasts growth factor (FGF) and insulin-like growth factor (IGF-1) play a role in increased proliferation. In addition, PRP and UCMSC can reduce the number of cells that experience apoptosis due to light radiation UVB, so it can provide the therapeutic potential for healing skin damaged by radiation UVB and oxidative stress, thus there will be an increase in the expression of total mRNA collagen and fibroblasts.⁸

Stem cells have two distinctive characteristics, namely: 1) Differentiation, in which stem cells can differentiate into specific cells; 2) Self-regenerate, which can renew or regenerate itself and make copies of cells like itself through cell division. Umbilical Cord Mesenchymal Stem Cell has shown to have the ability to secrete various secretions such as chemokines, cytokines, and growth factors. This series of secretome compounds serve as a mediator in communication between cells to repair and regenerate damaged tissue. This process is known as a paracrine effect. UCMSC is known to be able to repair damaged tissue through differentiation and paracrine effects. In some research results, it is suspected that UCMSC and its secretions have

anti-aging effects.⁹

However, PRP does not have properties like UCMSC, namely, the ability to differentiate into other cells, the ability to renew or regenerate itself, has a paracrine effect, and has the capacity for tumor homing. Therefore, UCMSC can be ascertained that it can produce more collagen and fibroblasts.

UCMSC was more effective than PRP in increasing amount of collagen and fibroblasts

In the previous research¹⁰, the mean collagen of Wistar rats (*Rattus norvegicus*) before being treated with UCMSC was 45.71%, and after the treatment increased to 57.22%. Moreover, the mean fibroblasts before treated with UCMSC was 17.78 cells/field of view and increased to be 24.86 cells/field of view after the treatment.¹⁰

The mean collagen before being treated with PRP was 44.32%, and after the treatment increased to be 53.41%, whereas the mean fibroblasts before treatment was 11.48 cells/field of view and increased to be 16.20 cells/field of view after the treatment.¹¹

In this study, the mean PRP collagen was 57.82%, and UCMSC collagen mean was 65.69%, while the mean PRP fibroblasts were 22.63 cells/field of view and the mean of UCMSC fibroblasts was 28.20 cells/field of view. Therefore, this study proved that the administration of UCMSC intradermal injection was more effective than PRP in increasing the amount of collagen and fibroblasts.

Conclusion

Based on the results of this study, it can be concluded that the administration of intradermal injection of UCMSC was more effective than PRP on the skin on the back of Wistar rats exposed to ultraviolet B rays and has been shown to increase the amount of collagen and fibroblasts.

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